

Genetic characterization of *Genista sericea* Wulfen (Cytiseae – Fabaceae) as revealed by nuclear DNA content and ITS nrDNA region analysis

LILIANA VIŽINTIN¹*, VERA KOSOVEL², LAURA FEOLI CHIAPELLA²,
BORUT BOHANEČ¹

¹ University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1111 Ljubljana, Slovenia

² University of Trieste, Department of Life Sciences, via Giorgieri 10, 34127 Trieste, Italy

Summary – *Genista sericea* Wulfen, a northern Illyrian amphi-Adriatic species, presents a certain morphological variability. To clarify whether the genetic variations support the morphological differences among accessions of different geographic origin, analysis of nuclear DNA content and polymorphism of the internal transcribed spacer (ITS) dataset was studied. The variation in nuclear DNA content of *G. sericea* var. *sericea* and var. *rigida* is minimal (2.09 and 2.08 pg/nucleus respectively) and is correlated with equal chromosome numbers in both varieties. Intraspecific variability of the ITS region was studied on 13 accessions of *G. sericea*, 6 belonging to var. *sericea* and 7 to var. *rigida*. These accessions were analyzed in comparison to closely related species already studied. ITS sequences of *G. sericea* revealed large polymorphism and formed two main clusters. One cluster (6 accessions) comprehends var. *sericea* of northern Italy, Slovenia and northern Croatia; the other cluster (7 accessions) includes five accessions of var. *rigida* from southern Croatia and Montenegro and two from the Pollino massif (southern Italy). The later two accessions considerably differed from other accessions of var. *rigida*. This genetic analysis supports the previous assumptions, which subdivided *G. sericea* into at least two taxa. On the basis of the results presented, it is here suggested that the subdivision of *G. sericea* into var. *sericea* and var. *rigida* should be maintained.

Key words: Fabaceae, *Genista sericea*, genome size, ITS, phylogenetic analysis.

Abbreviations: DAPI – 4, 6-diamidino-2-phenylindole, ITS – internal transcribed spacer, PI – propidium iodide.

Introduction

Genista sericea Wulfen, a northern Illyrian amphi-Adriatic species, has a range from northeastern Italy to Albania, with a disjunct distribution on the Pollino massif, Calabria, Italy (PAMPANINI 1912, GIBBS 1966, CONTI et al. 2005).

* Corresponding author, e-mail address: lili.vizintin@gmail.com

Copyright® 2012 by Acta Botanica Croatica, the Faculty of Science, University of Zagreb. All rights reserved.

Within the species, PAMPANINI (1912) distinguished var. *sericea* and var. *rigida*. In the first taxon the non-flowering branches are elongated and flexuous; the leaves are lanceolate and acute with a glabrous upper surface. In the second taxon the branches are shorter, more strongly ribbed and rigid; the leaves are ovate, broader and thicker, especially the young, with a sparsely pubescent upper surface. Whereas var. *sericea* is distributed in the northern part of the range (northeastern Italy and northern part of the Illyrian region), var. *rigida* is spread in the southern part of the Illyrian region (Dalmatia, Bosnia-Herzegovina, Montenegro, Albania) and on the Pollino massif (Italy). In recent literature, var. *rigida* is not considered (GIBBS 1966, PIGNATTI 1982, CONTI et al. 2005).

In northern Italy, var. *sericea* occurs along the southern slopes of the Alps; it is frequent in the east (Friuli Venezia Giulia, particularly in the karst area near Trieste), while it becomes rarer towards the west, in southern Veneto and southern Trentino. In Slovenia, var. *sericea* is present only in the south west. In Croatia it occurs in the northwestern area, especially in Kvarner (PAMPANINI 1912, PIGNATTI 1982, FEOLI CHIAPELLA and RIZZI LONGO 1987, CONTI et al. 2005).

Genista sericea belongs to sect. *Spartioides* Spach. According to its morphological and phytochemical characters, it is known as a heterogeneous section that does not form a monophyletic group. Because of the molecular data, the species of this section are included in separate groups intermingled with species of other sections and several intersectional hybrids are also present (PARDO et al. 2004). This heterogeneity was confirmed also by karyological and palynological data (CANTÓ et al. 1997; CUSMA VELARI et al. 2003, 2009; RIZZI LONGO and FEOLI CHIAPELLA 2009). The species of this section occur mostly in the Mediterranean region, with two main distribution centers: a Western (southern Spain and northwestern Africa) and an Eastern one (Balkan Peninsula and Anatolia) (GIBBS 1966, CANTÓ et al. 1997).

RIZZI LONGO and FEOLI CHIAPELLA (1993) carried out a pollen morphology analysis of *G. sericea* by light microscope and SEM, where var. *sericea* and var. *rigida* differ in some quantitative (grain dimensions) and qualitative (shape of amb and furrows) characters. CUSMA VELARI et al. (1996) have determined the chromosome number of *G. sericea* var. *sericea* and var. *rigida*: $2n=4x=48$.

The nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS) has proven a useful source of characters for phylogenetic studies in many Angiosperm families including Leguminosae (ÁLVAREZ and WENDEL 2003). Many genera of *Cytiseae* (KAESS and WINK 1997b), *Lupinus* (BADR et al. 1994, KAESS and WINK 1997a), *Genista* (DE CASTRO et al. 2002), *Genista* and related genera as *Teline*, *Echinospartum*, *Retama*, *Stauracanthus* (PARDO et al. 2004), *Cytisus* and allied genera such as *Argyrocytisus*, *Calicotome*, *Cytisophyllum* (CUBAS et al. 2002), *Adenocarpus*, *Genista* and *Teline* (PERCY and CRONK 2002) and *Ulex* (AINOUCHE et al. 2003), have already been analyzed.

Although molecular data are considered the most convincing data set, which can be used to identify phylogenetic relationships, a combination of different data sets, including genome size data, provides useful information on genetic similarity and taxonomic position (VIŽINTIN et al. 2006). Most reports (LEITCH and BENNETT 2002) confirmed that genome size is usually a value with limited variations within a single species and could be considered characteristic of the species. Flow cytometry could be used for estimations of genome size in absolute units using intercalating fluorochrome PI stained nuclei (NOIROT et

al. 2002) or for an intraspecific comparison in relative units using DAPI, an AT base specific dye (BUITENDIJK et al. 1997). Genome size data concerning the taxa *Genista*, *Cytisus*, *Lembotropis* and *Argyrocytistus* are rather scarce.

The aim of our study is to assess the intraspecific variability of *G. sericea*, highlighting the possible differences between var. *sericea* and var. *rigida* at a molecular level through the analyses of nrDNA regions ITS1 and ITS2. Furthermore, as genome size has not yet been determined for *G. sericea*, our aim is to provide data on genome size in both varieties.

Materials and methods

Plant material

Genome size determination was performed with fresh plant material. Accessions used for the flow cytometry analyses were: *G. sericea* var. *sericea*, collected at Opicina and Monrupino (Trieste – Italy) and *G. sericea* var. *rigida*, collected on the Pollino massif (Calabria, Italy) (Tab. 1).

Tab. 1. Propidium iodide and DAPI flow cytometric genome size determination in the examined populations of *Genista sericea*.

| Staining method | Taxon | Accession | No. plant | Average 2C (pg) ± SD |
|-----------------|---------------------------------------|---|-----------|----------------------|
| PI staining | | | | |
| | <i>G. sericea</i> var. <i>sericea</i> | Opicina (Trieste, Italy) 90579 (TSB) | 5 | 2.09 ± 0.03 |
| | <i>G. sericea</i> var. <i>rigida</i> | M. Pollino (Cosenza, Italy) 90582 (TSB) | 5 | 2.08 ± 0.03 |
| DAPI staining | | | | |
| | <i>G. sericea</i> var. <i>sericea</i> | Opicina (Trieste, Italy) 90579 (TSB) | 4 | 1.96 ± 0.02 |
| | | Monrupino (Trieste, Italy) 90580 (TSB) | 4 | 1.95 ± 0.02 |
| | <i>G. sericea</i> var. <i>rigida</i> | Pollino (Cosenza, Italy) 90582 (TSB) | 4 | 1.95 ± 0.03 |

The extraction of DNA for molecular analyses was performed from herbarium plant material. Different accessions of *G. sericea* were examined: six belonging to var. *sericea* and seven to var. *rigida*. The studied accessions, with their geographical origin and NCBI Genbank number are listed in table 2 and figure 1. Voucher specimens are deposited in the Herbarium of the Department of Life Sciences, University of Trieste, Italy (TSB). The classification of PAMPANINI (1912) was followed.

Determination of nuclear DNA content by flow cytometry

The relative and absolute value of DNA content was assessed by flow cytometry using *Trifolium pratense* L. as standard. The genome size (0.85 pg) was previously estimated for this species using *Trifolium repens* L. cv. Milo as standard (VIŽINTIN et al. 2006).

For the determination of absolute DNA content, tissues from green and young stems of the analyzed samples, together with leaf tissues of the standard species, were chopped with a razor blade in plastic Petri dishes in cold LB01 buffer, according to a technique adapted

Tab. 2. Accession data of the populations of *Genista sericea* sampled for phylogenetic analyses in the ITS region of nrDNA: origin, collector and NCBI GeneBank accession numbers.

| Taxon | Sample | Origin | Collector | GeneBank accession No. | |
|--|--------|--|--------------------|------------------------|----------|
| | | | | ITS1 | ITS 2 |
| <i>G. sericea</i> var. <i>sericea</i> | GS17 | Vivaro, Pordenone, Italy, 15/10/2000, 84731 (TSB) | L. Feoli Chiapella | EU525894 | EU525906 |
| | GS19 | Pinedo di Claut, Pordenone, Italy, 23/5/1999, 84728 (TSB) | C. Coran | EU525896 | EU525908 |
| | GS18 | Mt. Čaven, Nova Gorica, Slovenia, 10/5/1999, 84730 (TSB) | L. Feoli Chiapella | EU525895 | EU525907 |
| | GS20 | Mt. Nanos, Nova Gorica, Slovenia, 29/5/1999, 84726 (TSB) | L. Feoli Chiapella | EU525893 | EU525909 |
| | GS2 | Bašćanska Draga – Punat, Krk, Croatia, 24/4/1966, 84138 (TSB) | M. Tarabocchia | EU525897 | EU525910 |
| | GS15 | Capo Pax Tecum – Plomin, Istria, Croatia, 70 m, 26/4/1964, 84139 (TSB) | T. Mozenich | EU525898 | EU525911 |
| <i>G. sericea</i> var. <i>rigida</i> | GS12 | Biokovo, Kozica, Croatia, 600 m, 30/4/2001, 84457 (TSB) | L. Feoli Chiapella | EU525904 | EU525917 |
| | GS9 | Kuči, Donji Medun, Podgorica, Montenegro, 3/6/1998, 84143 (TSB) | V. Karaman | EU525900 | EU525913 |
| | GS10 | Kakarička gora, Masline, Podgorica, Montenegro, 173 m, 3/6/1998, 84456 (TSB) | V. Karaman | EU525901 | EU525914 |
| | GS11 | Piperi, Podgorica, Montenegro, 300 m, 17/5/1998, 90581 (TSB) | V. Karaman | EU525902 | EU525915 |
| | GS13 | Cijevna reka, Montenegro, 27/5/2000, 84451 (TSB) | S. Hadžiablahovič | EU525903 | EU525916 |
| | GS4 | Timpone Dolcetti, Pollino, Cosenza, Italy, 9/6/1997, 84141 (TSB) | D. Puntillo | EU525899 | EU525912 |
| | GS16 | Morano Calabro, Pollino, Cosenza, Italy, 3/6/1997, 84140 (TSB) | A. Vaccaro | EU525905 | EU525918 |

from DOLEŽEL et al. (1989). The suspension was passed through a 30 μm nylon-mesh filter and nuclei were stained with 50 $\mu\text{g mL}^{-1}$ of PI and 50 $\mu\text{g mL}^{-1}$ ribonuclease.

For the determination of relative DNA content, the staining was performed with DAPI and the procedure was modified according to OTTO (1988). Nuclei of our sample and of the standard species were released in 0.1 M citric acid containing 0.5% Tween 20. The suspension was filtered through a 30 μm nylon-mesh filter. A 4x volume of staining buffer containing 4 $\mu\text{g mL}^{-1}$ DAPI and 0.4 M disodium hydrogen phosphate was added.

Measurements were done on a Partec PAS flow cytometer using a linear scale. For PI staining, samples were analyzed using an argon laser tuned to 488 nm, with emissions measured through an RG 590 long-pass optical filter. For DAPI staining, the UV spectrum ex-



Fig. 1. Geographical origin of the examined accessions of *Genista sericea*. ▲ *G. sericea* var. *sericea*, ● *G. sericea* var. *rigida*

cited with a HBO lamp was used and emissions were measured through a GG 435 long-pass filter. Seven thousand nuclei per sample were measured and at least four repetitions of different nuclear isolations were performed for each species. Flomax® software (Partec, Münster) was used to calculate the positions of G0/G1 peaks of standard and investigated accessions. The analysis was completed in a short period to minimize the seasonal variation effect.

Analysis of the ITS region of rDNA

Genomic DNA was extracted from herbarium plant material applying CTAB mini DNA extraction protocol according to COMPTON et al. (1998) with slight modifications. The ribosomal ITS 1 and 5.8 S -ITS 2 regions were separately amplified using, respectively, ITS1-ITS2 and ITS3-ITS4 primers designated by WHITE et al. (1990). Amplification was carried out by means of a polymerase chain reaction in 50 µl reaction mixture containing 1 x Taq polymerase buffer (Promega, Madison, WI, USA), 200 µM of each dNTP, 0.5 µM of each primer, 100 ng DNA and 2.5 units of Taq polymerase (Promega). Optimized amplification was performed in a PTC-150-16E-25 M.J. Research Inc. thermal minicycler programmed as follows: (A) an initial 3 min denaturation at 95 °C; (B) 35 cycles of denaturation at 93 °C for 35 sec, annealing at 49 °C for 35 sec and polymerisation at 72 °C for 2 min; (C) final extension at 72 °C for 7 min. The amplified DNA products were sequenced from both sides by MacroGen Inc. (Seoul, Korea) using the same primers on an ABI 3730 XL DNA analyser (Applied Biosystems, Renton, USA). Sequence results from each amplified fragment were visualized and edited to obtain an optimized consensus sequence using CodonCode Aligner ver. 1.4.6 (CodonCode Corporation, USA). Nucleotide sequences were deposited in the GenBank database with the accession numbers reported in table 2.

Phylogenetic analysis of ITS sequences

The initial data matrix was aligned using ClustalX (ver. 1.83). A phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura 3-parameter model (TAMURA 1992) on Mega 5.0 software (TAMURA et al. 2011). Bootstrapping was performed at one thousand replicates to assess the confidence values of the clusters formed. Bootstrap data are shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 508 positions in the final dataset.

An accession of *Cytisus* was used as an outgroup. Several sequences of closely related *Genista* species from the section *Spartioides*, already available in the GenBank, have also been included in the ITS analyses to obtain a more comprehensive picture. Accession numbers and sources of these sequences are indicated in table 3.

Tab. 3. Accession data and sequences of ITS region obtained from NCBI GenBank.

| Taxon | References | GeneBank accession No. |
|--|-------------------|------------------------|
| <i>Genista cinerea</i> subsp. <i>speciosa</i> Rivas Mart. et al. | PARDO et al. 2004 | AY263637 |
| <i>Genista cinerea</i> subsp. <i>ausetana</i> O. Bolož s et Vigo | PARDO et al. 2004 | AY263638 |
| <i>Genista cinerea</i> (Vill.) DC. subsp. <i>cinerea</i> | PARDO et al. 2004 | AY263636 |
| <i>Genista majorica</i> Canto et M.J. Sanchez | PARDO et al. 2004 | AY263652 |
| <i>Genista ramosissima</i> (Desf.) Poir. | PARDO et al. 2004 | AY263662 |
| <i>Genista valentina</i> (Sprengel) Steud. | PARDO et al. 2004 | AY263676 |
| <i>Cytisus scoparius</i> (L.) Link subsp. <i>scoparius</i> | CUBAS et al. 2002 | AF351120 |

Results

Analysis of nuclear DNA content

Genome size values and base composition were determined for five plants of both varieties of *G. sericea* (var. *sericea* and var. *rigida*) using PI staining and for four plants of each accession of the two varieties using DAPI (Tab. 1). Determination using PI serves as an estimation of absolute nuclear DNA content, while determination using DAPI serves as a comparison for further intraspecific analysis. The absolute value of nuclear DNA content for var. *sericea* is 2.09 pg DNA per nucleus. The value obtained for var. *rigida* is 2.08 pg DNA per nucleus.

Because lower CV values cause higher resolution, DAPI staining was additionally used to test more accurately the intraspecific genome size variation: *G. sericea* var. *sericea* presents a relative nuclear DNA content of 1.95 – 1.96 pg per nucleus and var. *rigida* 1.95 pg per nucleus. Results obtained with DAPI staining confirmed that the variability of genome size is rather small between the two varieties of *G. sericea* and is not useful for the characterization of the varieties.

It should be noted that the values obtained by PI staining have given higher estimations of DNA content, suggesting unequal AT/GC content.

Polymorphism of the ITS region of rDNA

The internal transcribed spacer (ITS1 and ITS2) region of ribosomal DNA was sequenced from 13 accessions of *G. sericea* (Tab. 2). Each examined sequence produced a slightly different sequence profile; their alignment resulted in a matrix of 508 characters. A high variability of ITS nucleotide sequences was discovered exhibiting 22 variable sites.

The nucleotide sequences of amplified ITS regions were used to construct a phylogenetic tree (Fig. 2) to assess the relationships among the analyzed accessions. The ITS tree reveals two major clades, bootstrap support was 74. One clade is formed by all the accessions of var. *sericea*, from Friuli – Venezia Giulia (Italy), Slovenia and northern coast of Croatia. The other clade includes the accessions of var. *rigida* from southern Croatia coast and Montenegro. The two accessions of var. *rigida* from Pollino massif (southern Italy) stand at the base, but the bootstrap support is low.

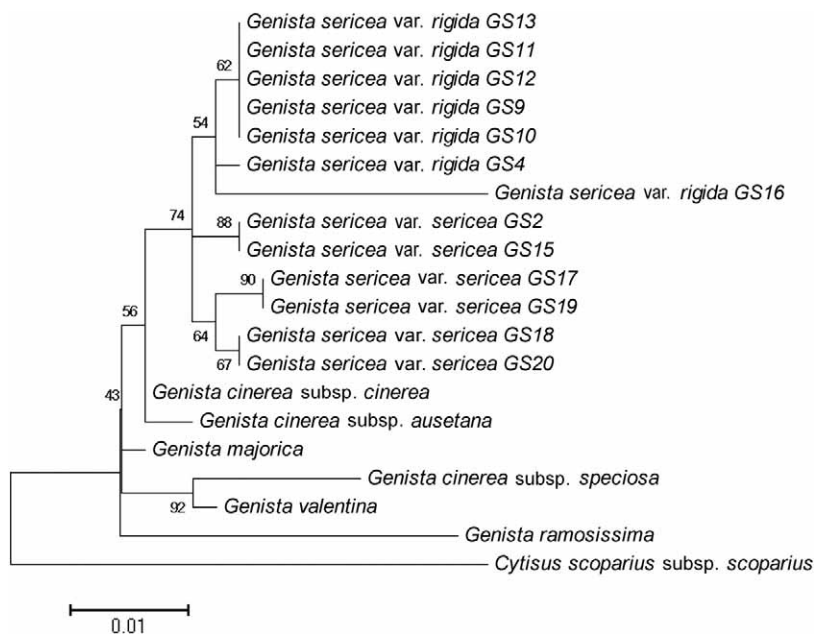


Fig. 2. Phylogram of 13 populations of *Genista sericea* and of 6 related species of the genus *Genista*. *Cytisus scoparius* subsp. *scoparius* was added as an outgroup.

Discussion

The accessions of *G. sericea* var. *sericea* and var. *rigida* present a very similar nuclear DNA content (2.09 and 2.08 pg per nucleus); also the relative nuclear DNA content is similar in the two varieties (1.95 – 1.96 and 1.95 pg per nucleus). The nuclear DNA content in a wild population of a species of *Genista sericea* is presented for the first time in this study, as the only data in literature regarding this genus are those of BELLENOT-KAPUSTA et al. (2006), who studied some ornamental brooms (as *G. tinctoria*, *G. lydia*, *G. pilosa*, *G. hispanica* and *G. aetnensis*) the 2C DNA content of which varies from 1.60 to 3.56, and of SUDA et al.

(2005) who determined the nuclear DNA content value for *G. benehoavensis* as 4.60 pg. In particular the 2C DNA content of *G. pilosa*, the only species previously examined of sect. *Spartioides*, is 1.99 pg, while in *G. tinctoria* and *G. lydia* of the affine sect. *Genista* is 1.73 and 1.74 pg. Data concerning other genera of *Cytiseae*, wild and cultivated plants of *Cytisus*, *Lembotropis*, *Argyrocytisus* and *Laburnum*, were published by BELLENOT-KAPUSTA et al. (2006) and OLSZEWSKA and OSIECKA (1983, 1984): the analyzed 2C DNA content varies from 1.10 to 4.40. MISSET and GOURRET (1996) determined the genome size of *Ulex* (7.7 pg/2C). OBERMAYER et al. (1999) and NAGANOWSKA et al. (2006) have studied many species of *Lupinus* (2C content from 1.15 to 2.68), a genus distant from the others of *Cytiseae*.

Because of the obtained genome size data, morphological differences between the two varieties of *G. sericea* are not correlated with significant differences in genome size and this led us to suppose that no major insertions or deletions of DNA segments occurred in any of varieties. This result is correlated with chromosome studies which showed the same number in both varieties ($2n=4x=48$; CUSMA VELARI et al. 1996).

Both varieties of *G. sericea* formed clearly separated clades (74 bootstrap supports) which did not intermingle with other related *Genista* species. Although analysis of ITS region is mainly used for interspecific phylogeny, this study indicates that genetic variation of ITS region in *G. sericea* was in this case sufficient to resolve both subspecies. In *G. sericea* the data presented reflect the separation of two distinct groups of accessions, the first comprehending all the accessions of var. *sericea* from northern Italy, Slovenia and northern Croatia, the second including all the accessions of var. *rigida* collected in southern Croatia, Montenegro and in southern Italy.

Molecular data are in accordance with morphological characteristics and pollen differences (RIZZI LONGO and FEOLI CHIAPELLA 1993), as well as seed dimensions (CUSMA VELARI and FEOLI CHIAPELLA, personal observations). Our analysis supports the subdivision of *G. sericea* into two taxa (var. *sericea* and var. *rigida*). On the basis of molecular data, the accessions of the Pollino massif (Calabria, Italy) attributed by PAMPANINI (1912) to var. *rigida* are related to those of southern Croatia and Montenegro, but do show diversification within var. *rigida* that could be further studied.

Acknowledgements

This research was supported by a grant from the Ministry of Foreign Affairs of the Italian Republic and in part by a project (P4-0077) of the Ministry of Higher Education, Science and Technology of the Republic of Slovenia.

We would like to thank Prof. L. Bernardo (Cosenza), Dr. D. Puntillo (Cosenza), Prof. A. Vaccaro (Acri), Dr. D. and V. Vincek (Kolašin) for supplying us with some plants and Dr. Lucia Feoli, for correcting the English version.

References

- AINOUCHE, A., BAYER, R. J., CUBAS, P., MISSET, M. T., 2003: Phylogenetic relationships within tribe Genisteae (Papilionoideae) with special reference to genus *Ulex*. In: KLITGAARD, B. B., BRUNEAU, A. (eds.), Advances in legume systematics, 10. Higher level systematics, 239–252. Royal Botanic Gardens, Kew.

- ÁLVAREZ, I., WENDE, J. F., 2003: Ribosomal ITS sequences and plant phylogenetic inference, *Molecular Phylogenetics and Evolution* 29, 417–434.
- BADR, A., MARTIN, W., JENSEN, U., 1994: Chloroplast DNA restriction site polymorphism in Genisteae (Leguminosae) suggest a common origin for European and American *Lupinus*. *Plant Systematics and Evolution* 193, 95–106.
- BELLENOT-KAPUSTA, V., PESTIL, C., CADIC, A., 2006: Diversity study and breeding of brooms (tribe of Genisteae). *Acta Horticulturae (ISHS)* 714, 29–36. <http://www.actahort.org/books/714/714>. Access June 11, 2012.
- BUITENDIJK, J. H., BOON, E. J., RAMANNA, M. S., 1997: Nuclear DNA content in twelve species of *Alstroemeria* L. and some of their hybrids. *Annals of Botany* 79, 343–353.
- CANTÓ, P., RIVAS-MARTÍNEZ, S., GREINWALD, R., VAN RENSEN, I., 1997: Revisión de *Genista* L. sect. *Spartioides* Spach en la península Ibérica y Baleares. *Lazaroa* 18, 9–44.
- COMPTON, J. A., CULHAM, A., GIBBINGS, J. G., JURY, S. L., 1998: Phylogeny of *Actaea* including *Cimicifuga* (Ranunculaceae) inferred from nrDNA ITS sequence variation. *Biochemical Systematics and Ecology* 26, 185–197.
- CONTI, F., ABBATE, G., ALESSANDRINI, A., BLASI, C., 2005: An annotated checklist of the Italian vascular flora. Palombi Editori, Roma.
- CUBAS, P., PARDO, C., TAHIRI, H., 2002: Molecular approach to the phylogeny and systematics of *Cytisus* (Leguminosae) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (trnL-trnF intergenic spacer). *Plant Systematics and Evolution* 233, 223–242.
- CUSMA VELARI, T., FEOLI CHIAPPELLA, L., KOSOVEL, V., 1996: Osservazioni cariosistematiche sul gruppo di *Genista sericea*. *Giornale Botanico Italiano* 130, 369.
- CUSMA VELARI, T., FEOLI CHIAPPELLA, L., KOSOVEL, V., 2003: Karyological notes on *Genista* sect. *Spartioides* Spach with emphasis on western species and *G. pilosa* L. (Genisteae-Fabaceae). *Studia Geobotanica* 22, 55–64.
- CUSMA VELARI, T., FEOLI CHIAPPELLA, L., KOSOVEL, V., 2009: Karyomorphology and systematics of the eastern taxa of *Genista* sect. *Spartioides* and *G. pulchella* (Genisteae-Fabaceae). *Caryologia* 62, 102–113.
- DE CASTRO, O., COZZOLINO, S., JURY, S. L., CAPUTO, P., 2002: Molecular relationships in *Genista* L. Sect. *Spartocarpus* Spach (Fabaceae). *Plant Systematics and Evolution* 231, 91–108.
- DOLEŽEL, J., BINAROVA, P., LUCRETTI, S., 1989: Analysis of nuclear DNA content in plant cells by flow cytometry. *Biologia Plantarum* 31, 113–120.
- FEOLI CHIAPPELLA, L., RIZZI LONGO, L., 1987: Distribuzione ed ecologia del genere *Genista* L. nel Friuli-Venezia Giulia. *Biogeographia* 13, 119–154.
- GIBBS, P. E., 1966: A revision of the genus *Genista* L. Notes from the Royal Botanic Garden, Edinburgh 27, 11–99.
- KAESS, E., WINK, M., 1997a: Molecular phylogeny and phylogeography of *Lupinus* (Leguminosae) inferred from nucleotide sequences of the rbcL gene and ITS 1+2 regions of rDNA. *Plant Systematics and Evolution* 208, 139–167.

- KAESS, E., WINK, M., 1997b: Phylogenetic relationships in the Papilionoideae (Family Leguminosae) based on nucleotide sequences of cpDNA (*rbcL*) and ncDNA (ITS 1 and 2). *Molecular Phylogenetics and Evolution* 8, 65–88.
- LEITCH, I. J., BENNETT, M. D., 2002: New insights into patterns of nuclear genome size evolution in plants. *Current Genomics* 3, 551–562.
- MISSET, M. T., GOURRET, J. P., 1996: Flow cytometric analysis of the different ploidy levels observed in the genus *Ulex* L. Faboideae-Genisteae in Brittany (France). *Botanica Acta* 109, 72–79.
- NAGANOWSKA, B., WOLKO, B., SLIWINSKA, E., KACZMAREK, Z., SCHIFINO-WITTMANN, M. T., 2006: 2C DNA variation and relationships among New world species of the genus *Lupinus* (Fabaceae). *Plant Systematics and Evolution* 256, 147–157.
- NOIROT, M., BARRE, P., LOUARN, J., DUPERRAY, C., HAMON, S., 2002: Consequences of Stoichiometric Error on Nuclear DNA Content Evaluation in *Coffea liberica* var. *dewevrei* using DAPI and Propidium Iodide. *Annals of Botany* 89, 385–389.
- OBERMAYER, R., SWIECICKI, W. K., GREILHUBER, J., 1999: Flow cytometric determination of genome size in some Old World *Lupinus* species (Fabaceae). *Plant Biology* 1, 403–407.
- OLSZEWSKA, M. J., OSIECKA, R., 1983: The relationship between 2C DNA content, life cycle type, systematic position and the dynamics of DNA endoreduplication in parenchyma nuclei during growth and differentiation of roots in some dicotyledonous herbaceous species. *Biochemie und Physiologie der Pflanzen* 178, 581–599.
- OLSZEWSKA, M. J., OSIECKA, R., 1984: The relationship between 2C DNA content, systematic position and the level of nuclear DNA endoreduplication during differentiation of root parenchyma nuclei in some dicotyledonous shrubs and tree. *Biochemie und Physiologie der Pflanzen* 179, 641–657.
- OTTO, F., 1988: High resolution DNA-flow cytometry using DAPI. Protocol, 1. Partec, Arlesheim, Münster.
- PAMPANINI, R., 1912: La *Genista sericea* Wulf. e la sua distribuzione in Italia. *Nuovo Giornale Botanico Italiano* 19, 327–348.
- PARDO, C., CUBAS, P., TAHIRI, H., 2004: Molecular phylogeny and systematics of *Genista* (Leguminosae) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (*trnL-trnF* intergenic spacer). *Plant Systematics and Evolution* 244, 93–119.
- PERCY, D. M., CRONK, Q. C. B., 2002: Different fates of island brooms: contrasting evolution in *Adenocarpus*, *Genista* and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. *American Journal of Botany* 89, 854–864.
- PIGNATTI, S., 1982: *Flora d'Italia* 1, 636–644. Edagricole, Bologna.
- RIZZI LONGO, L., FEOLI CHIAPELLA, L., 1993: Palynotaxonomy of *Genista sericea* group. *Proceedings 5th OPTIMA Meeting, Istanbul*, 745–760.
- RIZZI LONGO, L., FEOLI CHIAPELLA, L., 2009: Pollen morphology and taxonomy of *Genista* sect. *Spartioides* (Genisteae-Fabaceae). *Flora Mediterranea* 19, 141–160.
- SUDA, J., KYNCL, T., JAROLIMOVA, V., 2005: Genome size variation in Macaronesian angiosperms: forty percent of the Canarian endemic flora completed. *Plant Systematics and Evolution* 252, 215–238.

- TAMURA, K., 1992: Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* 9, 678–687.
- TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M., KUMAR, S., 2011: MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.
- VIŽINTIN, L., JAVORNIK, B., BOHANEK, B., 2006: Genetic characterization of selected *Trifolium* species as revealed by nuclear DNA content and ITS rDNA region analysis. *Plant Science* 170, 859–866.
- WHITE, T. J., BRUNS, T., LEE, S., TAYLOR, J., 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M. A., GELFAND, D. H., SNINSKY, J. J., WHITE, T. J. (eds.), *PCR protocols: A guide to methods and applications*, 315–322. Academic press, San Diego.